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The multifunctional protein PEA-15 is involved in the control of apoptosis and cell cycle in astrocytes

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Abstract

PEA-15 is a small protein (15 kDa) that was first identified as an abundant phosphoprotein in brain astrocytes [Araujo *et al.*, J Biol Chem 1993;268(8):5911–20], and subsequently shown to be widely expressed in different tissues and highly conserved among mammals [Estelles *et al.*, J Biol Chem 1996;271(25):14800–6; Danziger *et al.*, J Neurochem 1995;64(3):1016–25]. It is composed of a N-terminal death effector domain and a C-terminal tail of irregular structure. PEA-15 is regulated by multiple calcium-dependent phosphorylation pathways that account for its different forms: a non-phosphorylated form in equilibrium with a mono and a biphosphorylated variety. This already suggested that PEA-15 may play a major role in signal integration. Accordingly, it has been demonstrated to modulate signaling pathways that control apoptosis and cell proliferation. In particular, PEA-15 diverts astrocytes from TNFalpha-triggered apoptosis and regulates the actions of the ERK MAP kinase cascade by binding to ERK and altering its subcellular localization. The three-dimensional structure of PEA-15 has been modelized and recently determined using NMR spectroscopy, and may help to understand the various functions played by the protein through its molecular interactions.

Keywords: PEA-15; MAP kinases; TNFalpha; Death effector domain; Calcium-dependent kinases

1. Introduction

During the last two decades, multiple functions have been assigned to astrocytes, among which their capacity to initiate dynamic responses when stimulated *in vivo* by a wide variety of extracellular signals. An important cellular response consists in increases in intracellular calcium that influence many astrocytic functions, ranging from cytoskeletal rearrangement to intercellular communication through calcium waves. Intracellular phosphoproteins can be considered as targets for extracellular signals received by the cell. Their phosphorylation modifies their function and consequently some of the cell properties. The high expression in astrocytes of a small acidic (pI 5.2–5.4) phosphoprotein, that we named PEA-15, led us to explore the function it played in these glial cells.

2. PEA-15 phosphorylation is tightly regulated

PEA-15 exists *in vivo* as three isoforms, namely N, Pa and Pb, which correspond to the unphosphorylated, mono and diphosphate forms, respectively. Phosphorylation occurs on two seryl residues. The first one, Ser104, is located within the motif LTRIPSAKK, is a PKC target [1]. The second site, Ser116, is included in the motif DIRQPSEEEIIK, and is the target of the CaMKII [4]. 2D-SDS–PAGE analysis of ^{32}P-labeled proteins extracted from astrocytes exposed to neurotransmitters or growth factors, showed that phosphorylation of Ser104 and Ser116 are tightly regulated. For instance, the norepinephrine-induced activation of α1-adrenergic receptors, resulted in PEA-15 phosphorylation by PKC [1]. Similarly, endothelin isoform

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Abbreviations: DED, death effector domain; PKC, protein kinase C; ERK MAP kinase, extracellular regulated kinase mitogen-associated protein kinase; NMR, nuclear magnetic resonance; TNFalpha, tumor necrosis factor alpha; CaMKII, calcium–calmodulin-dependent kinase type II; FADD, Fas-associated death domain; PLD1/PLD2, phospholipase D1 and D2.

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ET1, known to promote a large and prolonged increase in intracellular calcium levels in astrocytes [5], markedly enhanced PEA-15 phosphorylation and did so through both PKC-dependent and independent pathways. Indeed, ET1 still stimulated PEA-15 phosphorylation when the activation of PKC was prevented either by inhibiting the enzyme with a low concentration of staurosporine or following desensitization of the kinase with TPA. In these conditions, two-dimensional peptide mapping and microsequencing allowed to demonstrate that Ser116 is the site phosphorylated by CaMKII in intact astrocytes stimulated by ET1. In addition, a hierarchal phosphorylation was evidenced as Ser116 phosphorylation by CaMKII greatly enhanced the ability of PKC to phosphorylate Ser104 [4].

Protein phosphatases are still poorly characterized in astrocytes. Membrane-permeant phosphatase antagonists were therefore used to determine the PEA-15 dephosphorylation pathway: okadaic acid (OK) that inhibits phosphatase 2A (PP2A) and, with a lesser efficacy, phosphatase 1 (PP1), Calyculin A (CalA) which is equipotent to inhibit PP1 and PP2A, and Tautomycin (Tau) that preferentially inhibits PP1 [6]. PEA-15 phosphorylation was rapidly enhanced following treatment of the striatal astrocytes with OKand CalA whereas Tau was poorly effective. These results suggest that PP2A is essentially involved in PEA-15 dephosphorylation. Furthermore, high resolution 2D-SDS–PAGE analysis and microsequencing revealed that Pa is a combination of two sub-isoforms: Pa1, solely phosphorylated on Ser116, and Pa2, phosphorylated only on Ser104 and essentially resulting from the dephosphorylation of Ser116 within Pb.[1]

3. PEA-15 inhibits TNFalpha-induced apoptosis

Programmed cell death of neurons and oligodendrocytes has been extensively documented in several pathological conditions. On the contrary, the astrocytes respond to any brain injury in a process called reactive gliosis. They appear to be relatively resistant to central nervous system damage and are at the core of tissue repair and regeneration at the site of injury [7]. The pro-apoptotic cytokine TNFalpha expression is up-regulated in reactive astrocytes [8], which also express apoptosis-inducing receptors belonging to the TNF receptor superfamily, such as TNFR1 or Fas. They are not, however, susceptible for example to Fas-mediated cytotoxicity [9], suggesting the existence of a self-protective mechanism.

TNF triggers multiple responses in astrocytes, including cell proliferation, upregulation of TNF mRNAs, production of interleukin 8, macrophage-, granulocyte- and granulocyte-macrophage colony stimulating factors (reviewed in [10]). In addition, TNF primes astrocytes to render them immunocompetent through the induction of the expression of several surface molecules including MHC class II molecules, ICAM-1, VLA-1 and -2, allowing the recruitment of lymphocytes and monocytes [8]. Considering the time course of these events, astrocytes are likely to need protection against the pro-apoptotic effect of TNF and other inflammatory factors, and PEA-15 may play an important role in this process.

Membrane receptor-induced apoptosis results from an orderly cascade of cellular events. Upon binding to their respective ligands, Fas and TNFR1 receptors, FasL or TNF initiate apoptosis by recruiting the cytosolic adaptor molecule FADD to the plasma membrane to form a multiprotein complex named death inducing signaling complex (DISC) (Fig. 1A) (reviewed in [11,12]). The N-terminal part of FADD contains a DED that binds to homologous domains located in the N-terminal part of caspase-8 allowing the activation of the caspase [13,14]. Mutants of FADD lacking DED, or mutants of caspase-8 with only its DEDs can act as dominant-negative inhibitors suggesting that endogenous inhibitors of the early steps of apoptosis could exist.

PEA-15 also contains a DED. We demonstrated that PEA-15 interacts *in vitro* with two other DED domain containing proteins, FADD and caspase-8 [15] (Fig. 1A). PEA-15 molecules in the proximity of caspase-8 and caspase-10 in the DISC may prevent further cleavage of the caspases according to the induced proximity model. Indeed, PEA-15 can be recruited in the DISC [16]. Furthermore PEA-15 expression is required to divert astrocytes from the deleterious effects of TNF. This was demonstrated using astrocytes from wild type versus PEA-15 null mutant mice [15]. Astrocytes lacking PEA-15 and exposed to TNF rapidly exhibited the classical signs of apoptosis including inversions of membrane lipids, evidenced by annexin V labeling, and nuclear fragmentation leading to the formation of DNA ladders [15]. Re-expression of PEA-15 after transfection restored protection and survival.

In vitro interaction of PEA-15 with FADD and caspase-8 appeared weaker than that observed between FADD and caspase-8 [15], suggesting that post-translational modifications are important *in vivo*. Indeed, in astrocytes PEA-15 is essentially present under its phosphorylated forms [1,3]. The role of PEA-15 phosphorylation to prevent apoptosis has been latter demonstrated in other cellular models. NIH3T3 cells transfected with wtPEA-15 are protected from Fas-induced apoptosis whereas a double mutant S104A/S116A is inactive [2]. Furthermore, it was recently reported that PEA-15 is recruited to the DISC after TRAIL stimulation [16]. It appears that PKC-dependent PEA-15 phosphorylation modulates its antiapoptotic functions. PKC inhibition restores TRAIL sensitivity in TRAIL-resistant cells [17]. The same group further showed that treatment with KN-93 (a CaMK inhibitor) also largely rescues the cells sensitivity to TRAIL [16]. Interestingly, a doubly phosphorylated form of PEA-15 is recruited to the DISC in TRAIL-resistant cells. Furthermore, doubly phosphorylated PEA-15 proteins are expressed in TRAIL-resistant

[1] Malvyne Rolli-Derkinderen and Herve Chneiweiss, unpublished results.

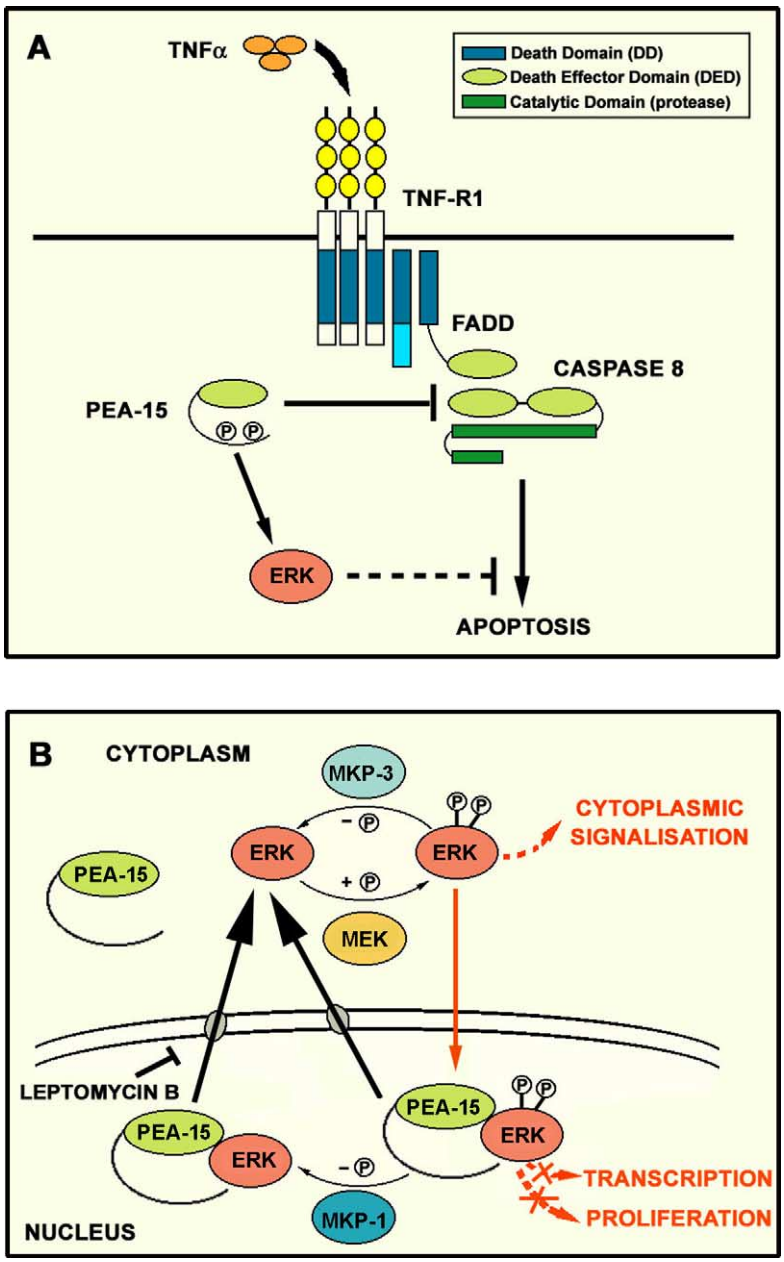

Fig. 1. PEA-15 regulates both TNFalpha-induced apoptosis and ERK-dependent transcription. (A) PEA-15 participates in the formation of the DISC formed of the receptor, FADD, caspase-8 as mentioned in the text, where it may bind to FADD and/or pro-caspase-8, blocking the activation of the caspase. An indirect effect on ERK (dotted line) may also participate in the anti-apoptotic effect of PEA-15. (B) PEA-15 binds and exports ERK from the nucleus, redirecting the kinase activity toward its cytosolic substrates.

but not TRAIL-sensitive cells, further indicating a significant difference in the upstream mechanisms that control phosphorylation of PEA-15 in TRAIL-resistant cells compared with TRAIL-sensitive cells [16,18].

4. PEA-15 limits entry into the cell cycle

PEA-15 is particularly enriched in mature, non-dividing astrocytes. During development of the brain, PEA-15 expression begins relatively late at embryonic day 12 (E12) [3] and reach the adult levels by postnatal day 10 (P10) when cell proliferation is strongly reduced in the CNS. Moreover, the levels of PEA-15 in proliferating astrocytes are lower than in non-proliferating astrocytes in cultures [3]. Conversely, PEA-15 null mice exhibit a marked increase in cell proliferation in several lineages including astrocytes, lymphocytes and hepatocytes [19].[2] Thus, PEA-15 expression appears closely, and inversely, correlated with cell proliferation.

The ERK1/2 p44/42 ERK MAP kinase cascade seems essential for entry into the cell cycle (reviewed in [20]).

[2] Le Gouvello S, Beuvon F and Chneiweiss H, unpublished results.

Multiple substrates of ERK1 and ERK2 have been characterized, located in different subcellular compartments such as the plasma membrane for the epidermal growth factor receptor, the cytosol for the microtubule destabilizing protein stathmin, or the nucleus for the transcription factor Elk1. The inactive ERK is essentially located in the cytosol. Its activation leads to its translocation into the nucleus, an event that seems to be essential for ERK action and signal termination [20]. The first evidence for a link between PEA-15 and the MAP kinase signaling pathway came from the reversal of the H-Ras inhibition of integrin signaling by PEA-15 expression, evidenced using expression cloning [21]. Surprisingly, PEA-15 blocked Ras effect on integrin without affecting its stimulation of ERK activity. We subsequently demonstrated a direct interaction between PEA-15 and the MAP kinase cascade after a yeast two-hybrid screen, using PEA-15 as a bait, that allowed to characterized ERK1 and ERK2 as the PEA-15 partners [19].

PEA-15 is not a substrate for ERK. Indeed, PEA-15 lacks the canonical proline-directed ERK phosphorylation sites [22] and is not phosphorylated by ERK *in vitro*. PEA-15 alters the output of ERK signaling without blocking ERK activation. Indeed, we observed that PEA-15 does not interfere with ERK activation or activity. PEA-15 does not affect the phosphorylation of ERK cytosolic substrates, such as stathmin or p90RSK, but it blocks the phosphorylation of ERK nuclear substrates, such as Elk-1, with a consequent inhibition of ERK-dependent transcription.

PEA-15 modifies ERK signaling by excluding ERK from the nucleus (Fig. 1B). High expression of PEA-15 results in export of ERK from the nucleus in astrocytes, and in PEA-15 transfected CHO or NIH3T3 cells. Moreover, we found that the physiological levels of PEA-15 expressed in cultured astrocytes are sufficient to restrict ERK to the cytoplasm, and to block ERK-dependent cFos transcription and cell proliferation. PEA-15 contains a nuclear export signal (NES) that is required for ERK localization to the cytosol. Leptomycin B, a specific inhibitor of CRM1 (exportine-1/chromosome region maintenance 1)-mediated nuclear export, caused PEA-15 accumulation in the nucleus. The NES within PEA-15 is located in I15L17 and a mutation of this sequence results in both PEA-15 and ERK nuclear localization [19]. The ERKs may be inactivated by exposure to phosphatases, such as MKP-1, in the nucleus. Thus, a rapid export from the nucleus could contribute to the capacity of PEA-15 to potentiate ERK activation [23]. Hence, changes in the expression levels of PEA-15 can redefine cellular responses to the ERK MAP kinase pathway by excluding ERK from the nucleus.

5. Additional functions of PEA-15

Using differential display to identify genes whose expressions are altered in tissues derived from type II diabetes mellitus patients compared with nondiabetic individuals, Condorelli *et al.* [24] cloned cDNAs encoding PEA-15, which they named PED for "phosphoprotein enriched in diabetes". They found that PEA-15 mRNA was overexpressed in fibroblasts, skeletal muscle, and adipose tissue from type II diabetics. The protein levels were also elevated in type II diabetic tissues. Furthermore, transfection of a PEA-15 cDNA into differentiating L6 skeletal muscle cells increased the content of glucose transporter-1 (GLUT1) on the plasma membrane and inhibited insulin-stimulated glucose transport and cell surface recruitment of glucose transporter-4 (GLUT4). These effects were reversed by the inhibition of PKC activity, suggesting that the phosphorylation of PEA-15 might be involved. While the role of ERK in glucose transport is uncertain, based on our data it is reasonable to predict that PEA-15 overexpression could block glucose stimulated ERK translocation [25]. This may partly explain the changes observed in glucose transport and cell surface recruitment of GLUT4 in type II diabetes.

A two-hybrid search also evidenced an interaction of PEA-15 with PLD1, that was further confirmed in intact cells [26]. Elevated levels of PLD1 activity were observed *in vitro* and *in vivo* after co-expression with PEA-15. This enhanced PLD1 activity was correlated with elevated levels of PLD protein, leading to the conclusion that PEA-15 was affecting the accumulation or degradation rates of PLD1 rather than its activity *per se*. Frohman and co-workers [31] also hypothesized that PEA-15 could function as a chaperon to interact with the C-terminal region of PLD to facilitate its folding and/or transport to the membrane. A protein that does not fold correctly is targeted for quick elimination; hence, the presence of PEA-15 could lead to higher steady-state levels of PLD1 expression.

Formation of a functional PLD1-interacting site on PEA-15 requires the C-terminus of PEA-15 and part of its DED. Binding of PLD1 to PEA-15 might recruit PLD1 into the complexes that initiate apoptosis through simultaneous interaction with FADD/caspase-8 and PLD1. This hypothesis is attractive since PEA-15 and PLD1 have both been proposed to have roles in apoptosis [2,15,27,28]. Moreover, caspase-8 (but not FADD) exhibits sequence similarities with PEA-15 that extends beyond the DED domain through the length of the C-terminal PLD1-interacting region. This suggests that caspase-8 and related family members might also exhibit affinity for PLD1 or PLD2 [26].

Astrocytes are responsible for the major part of the central nervous system PLD response to signaling agonists such as endothelin [29]. Interestingly, PLD1 and PLD2 have been linked at several levels, including the receptor, to insulin-signaling and glucose transport including at the receptor level. Most relevant, is a very recent report that PLD1 and Glut4 co-localize and that increasing PLD activity promotes translocation of Glut4 to the plasma membrane [30]. Since PEA-15 overexpression reportedly decreases Glut4 translocation to the plasma membrane, one interpretation might be that the excess PEA-15 sequestered or diverted PLD1 away from the Glut4 pathway.

Finally, PEA-15 may recruit PLD to other receptor complexes through the same mechanism. Indeed, PLD2 and PEA-15 co-immunoprecipitate with the EGF and insulin receptors,[3] suggesting that PEA-15 and PLD2 are associated in this setting.

6. The *pea15* gene and its transcription

We cloned two forms of mouse PEA-15 cDNA that differ in the length of the 3′UTR. These likely represent transcripts generated by alternative polyadenylation [2]. Northern blots indicate that expression of PEA-15 is predominant in the CNS. However, *pea15* transcripts are also detected in several peripheral organs. This suggests additional functions for PEA-15 required in multiple cells and tissues, beside its specific role in astrocytes.

The *pea15* gene has now been cloned in several mammals and found to be highly conserved. The protein differs only by one conservative amino acid change between human, mouse, rat, chinese hamster and ox. Despite the fact that several antibodies directed against different parts of the molecule recognize a band around 15 kDa in birds and fish [3], it was not possible to clone the corresponding cDNAs. Furthermore, computer analysis of the drosophila genome did not reveal any sequence related to *pea15*. Thus, it might be possible that *pea15* only appeared with mammals.

The genomic sequence of *pea15* is composed of four exons and spans approximately 10.2 kb of genomic DNA flanked upstream by a potentially expressed Alu element and downstream by the H326 gene [32]. The human *pea15* gene was mapped to 1q21–q22, between the markers D1S2635 and D1S484 [33]. A few diseases were mapped to this region, including type II diabetes in Pima Indians, autosomal dominant non-syndromic hearing impairement locus, *DNFA7*, hyperparathyroidism-jaw syndrom, familial hemiplegic migrane, *MHP2*, a non-Hodgkin lymphoma [34] and the loop–tail mutation in mouse. A detailed gene scan didn't found any evidence for a *pea15* mutation in type II diabetes or loop–tail mutation [32,34].

In addition to the remarkable conservation of the PEA-15 protein sequence, three highly conserved regions are found within the 3′UTR of its cDNAs, each greater than 100 nucleotides in length. 3′UTRs are known to contain regulatory sequences that signal mRNA localization, translational regulation and direct degradation. Conserved sequences found in mouse and human PEA-15 cDNA 3′UTR are good candidates for such roles. Indeed, several infrequent regulatory motifs were found in these regions, including JCV repeats. The human JC polyomavirus (JCV) is the etiologic agent of the neurodegenerative disease progressive multiple leukoencephalopathy, and replicates only in astrocytes. Several studies have established that the restricted host range of JCV to glial cells is determined at the level of viral transcription, which is mediated by glial-enriched DNA-binding regulatory proteins [35].

In good agreement with an important function of PEA-15 3'UTR, is the presence within this region of the proto-oncogene MAT1. Indeed, we demonstrated that MAT1 cDNA is a partial sequence of the PEA15 2.4 kb mRNA and does not encode a protein [2]. The MAT1 sequence was isolated from a mouse mammary tumor induced *in vitro* with *N*-methyl-*N*-nitrosourea and lithium, and was reported to induce the oncogenic transformation of NIH-3T3 cells [36]. Accordingly, increased expression of PEA15 transcripts was reported in numerous tumors, including gliomas, the main primary tumors of the brain (for a detailed list, see pea15 in the OMIM database). This was particularly the case for oligodendrogliomas, with and without 1p/19q loss. PEA15 mRNA expression was also reported in other cancers such as ovarian, kidney or hepatocellular carcinoma, lymphomas, or melanomas. PEA-15 mRNA expression levels were higher in breast cancer cell lines than in normal mammary epithelial cells [33]. As previously mentioned, a genetic deletion of PEA-15 results in astrocytes that are more proliferative. Thus, two hypothesis might explain the oncogenic role of the 3′UTR: either a trapping of regulatory molecules, or a decrease in the level of the protein expressed in the cell.

Finally, in addition to the previously reported up-regulation of the protein PEA-15 essentially after birth, PEA-15 mRNAs expression was found increased in several conditions. Using multiplex RT–PCR, Glienke *et al.* [37] reported an overexpression of PEA-15 mRNAs in human endothelial cells MVEC cultured on matrigel. This adhesive matrix stops MVEC proliferation and induces cells to form capillary-like structures mimicking early steps of angiogenesis. Studying Pre-B to immature B cell transition with DNA microarrays, Li *et al.* [38] found a 12-fold increase in PEA-15 after NF-κB stimulation.

7. PEA-15 structural properties

The DED is one of several small protein recognition modules that mediate the assembly of complexes required for signal transduction in programmed cell death. DEDs found in the adaptor protein FADD and the proform of the initiator caspases, caspase-8, play a pivotal role in the initiation of death receptor-mediated apoptosis, whereas DEDs in viral or cellular FLICE-inhibitory proteins (FLIPs) have the ability to block apoptosis [39]. The DED, together with the structurally related death domain (DD), caspase recruitment domain (CARD), and more recently recognized pyrin domain (PYD) are members of the death motif superfamily characterized by a conserved six α-helix bundle structure [40,41]. In addition to a common three-dimensional (3D) fold, these protein domains typically associate via homotypic interactions with complementary domains in their binding partners.

[3] Frohmann M, unpublished data and [31].

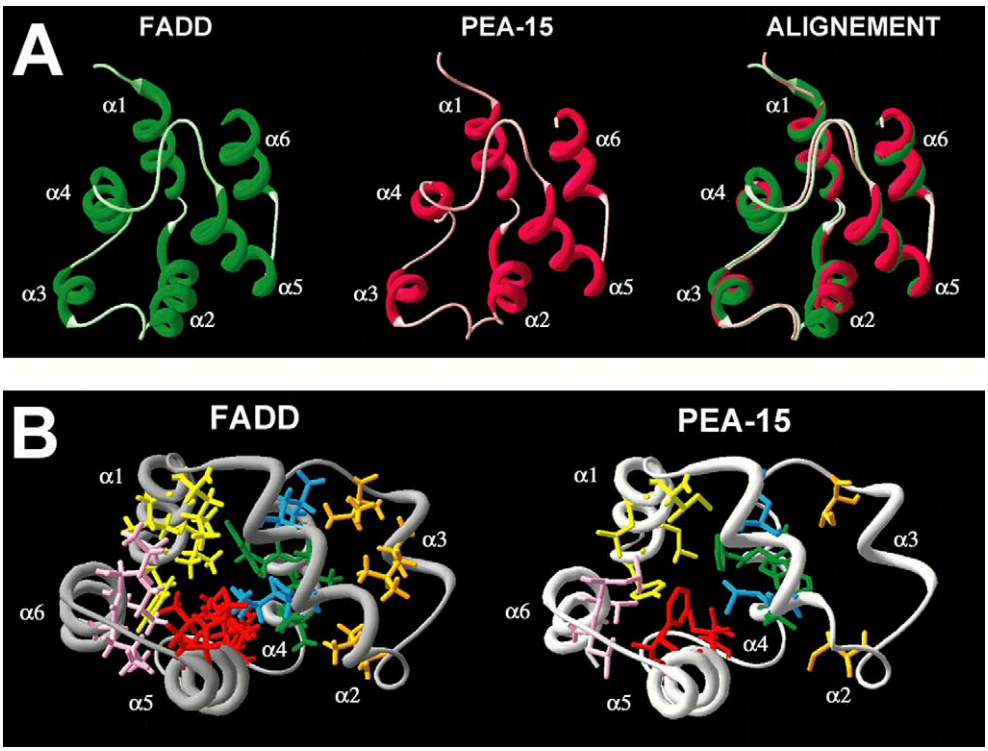

Fig. 2. Computerized modelization of PEA-15 three-dimensional structure. (A) Comparison of the DED of PEA-15 and FADD evidences a similar general structure made of six antiparallel α-helices forming a Greek key. (B) Ribbon representation of FADD and PEA-15 DEDs. The hydrophobic residues point to the core of the molecule. Seventeen out of 19 hydrophobic residues located in the core of FADD have a similar location within PEA-15. Colors distinguish the helices: α1, yellow; α2, blue; α3, orange; α4, green; α5, red; α6, pink.

The observation of an interaction between PEA-15 DED and ERK1/2 [19,23] suggested a greater functional versatility for this structural motif. The 3D structure of PEA-15 was recently determined using NMR spectroscopy and its interaction with ERK2 established from an *in vitro* and *in vivo* analysis of PEA-15 mutant proteins [42]. PEA-15 consists of an N-terminal DED comprised of six antiparallel amphipathic α-helices closely packed around a central hydrophobic core, followed by a long C-terminal tail (Fig. 2A). The C-terminal tail is irregular in structure, with the exception of residues 120–123, which appear to form a single turn of a 310°-helix. The α-helices in the DED are connected by short loops, two of which contain β-turns (α2–α3 and α4–α5). The α-helices are arranged in a Greek key topology, with helices α1 and α2 being centrally located, α3 and α4 on one side and α5 and α6 on the other. This fold represents the core structure of the death motif superfamily [40]. A new PEA-15 mRNA spliced form was recently identified in E15 mice embryos [43], lacking 22 amino acids mostly representing α3 and α4. This variant, which remains to be characterized at the protein level, should lack an entire side of the Greek key but may keep the overall folding.

The overall fold of the PEA-15 DED closely resembles the structure of the FADD DED (Fig. 2B). However, several essential differences exist. Helix α6 of PEA-15 is seven residues longer than that of the FADD DED, and on the other hand the N-terminus of the FADD DED is oriented away from α6 and the core of the protein (Fig. 2). PEA-15 also lacks the two hydrophobic patches observed on the surface of FADD DED, including Y25 located in helix α2 that has been implicated in FADD's apoptotic activity and interaction with the DEDs of procaspase-8 [44].

The α5–α6 loop contains a conserved RxDLf motif (x: any amino acid; f: any hydrophobic residue) that is found in many DED-containing proteins. Garvey *et al.* [45] demonstrated that the α1–α2 and α5–α6 loops of both DEDs in viral FLIP MC159 are important for its ability to inhibit apoptosis via interaction with FADD and procaspase-8. Interestingly, a PEA-15 mutant, D74A, failed to bind FADD and caspase-8.[4] Furthermore, the same mutant also failed to bind ERK and did not prevent ERK nuclear translocation or ERK-dependent transcription or cell proliferation.

The D74A mutation is in the last few amino acids of the DED domain adjacent to the C-terminal half of the protein, and neither the C-terminus nor the DED is sufficient for ERK binding. This suggests that an extended surface of PEA-15, which involves both DED and C-terminal domains, is required for ERK binding. Supporting this conclusion, the canonical DED-protein FADD does not bind ERK, indicating that ERK may specifically interact with PEA-15 amongst the DED-containing molecules. Finally, both the DED and the C-terminus parts are also required for PEA-15 binding to PLD1, which does not bind

[4] Canton B and Chneiweiss H, unpublished results.

FADD or caspase-8 further indicating the specificity of PEA-15 folding.

Previously identified ERK binding proteins fall into three classes: (i) scaffolding proteins such as MP1; (ii) enzymes directly activating or inactivating ERK such as MEK and the phosphatases MKP-3, PTP-SL and STEP; (iii) and docking proteins associated with the microtubule cytoskeleton such as MAP2 (reviewed in [20]). There is no obvious sequence similarity between PEA-15 and other known ERK binding proteins. Moreover, PEA-15 does not appear to be a scaffold protein since it does not bind other constituents of the ERK pathway. Furthermore, PEA-15 lacks proline-directed ERK phosphorylation sites [22] and it is not a substrate for ERK *in vitro*. Finally, PEA-15 does not contain a D-domain, which is an ERK MAP kinase binding sequence found in proteins such as Elk-1. Hence, PEA-15 may define a new class of ERK-binding proteins.

8. Conclusion

PEA-15 appears as a multifunctional adaptor molecule able to modulate the outcomes of the DISC as well as of the ERK MAP kinase cascade. Essentially expressed in mature non-dividing cells, it plays a ''double-key'' role: promoting survival and limiting proliferation, both functions being regulated by calcium-dependent phosphorylations. Further investigations will help to understand whether the additional partners such as PLD1 and tyrosine kinase receptors participate in already identified functions of PEA-15 or are involved in other types of cell regulation.

Acknowledgments

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